

## **FINAL REPORT**

### **NOAA Award NA05OAR4311162**

**Title:** Does the ice-covered Southern Ocean act as a “dissolved organic matter pump” to the abyssal ocean and so sequester carbon from active cycles?

**Project Duration:** 1 June 2005 – 31 May 2009

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**Institution:** Florida State University

### **Introduction:**

Dissolved organic matter (DOM) in the oceans contains about the same amount of carbon as the global biomass or atmospheric CO<sub>2</sub> and exhibits an average age of several thousand years. Source, diagenesis and preservation mechanisms of DOM remain elemental questions in contemporary marine sciences and represent missing links in models of global elemental cycles. The polar oceans are probably a primary source of DOM to the deep ocean because these regions are the only places where surface waters efficiently convect down to the oceans' bottom. Deep-water formation is directly linked to sea-ice formation, when salt is rejected and dense brine-enriched waters penetrate the deep ocean. Sea ice is one of the most productive marine environments, and DOM concentrations in the brine are among the highest measured in marine waters. The biogeochemistry of sea ice is largely unknown and it is not clear whether sea-ice DOM is persistent enough to survive downward convection. The Weddell Sea off Antarctica is the prime site for bottom water formation. We hypothesize that significant amounts of DOM may be exported from the surface Weddell Sea into the abyssal ocean and globally distributed via the thermohaline circulation. Quantitative knowledge of this process is vital for our understanding of the global carbon cycle and future climate changes.

### **Project Goals:**

The objective of this project was to investigate the formation of persistent DOM in sea ice and to trace its fate into the deep sea. Broad significance related to answering the question: *Do ice-covered oceans act as a “DOM pump” to the abyssal ocean and so sequester carbon from active cycles?* The project's primary goal was to provide the first quantitative estimates on the amount of carbon (Tg) annually sequestered by this process in the Weddell Sea and in ice-covered oceans worldwide.

### **Methods:**

#### *Sampling:*

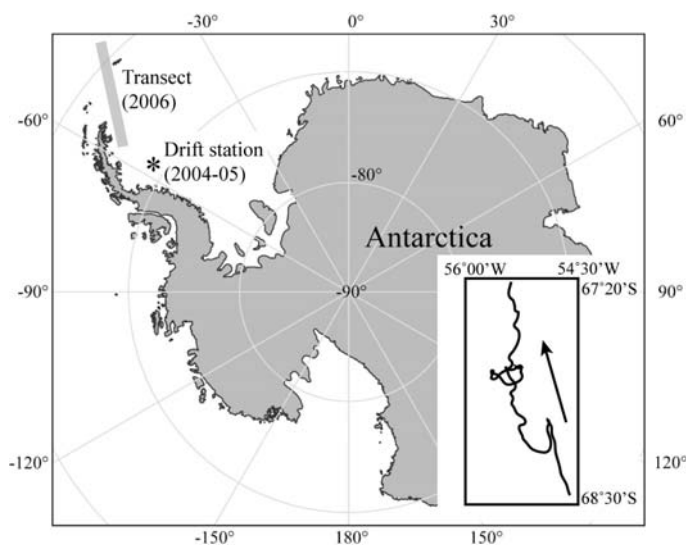
Fieldwork and sampling in the Weddell Sea (Antarctica) was performed within the multinational, interdisciplinary field experiment ISPOL (Ice Station POLarstern) with support of the German Icebreaker RV Polarstern (Alfred Wegener Institute for Polar and Marine Research). From Nov. 28, 2004 to Jan. 2, 2005 RV Polarstern was anchored to an ice floe at 68°S/55°W where most samples were taken (Figure 1). Approximately 200 experimental and in situ samples from different

sources were taken, including detailed ocean column profiles, ice, and brine. For further molecular characterization, DOM was isolated via solid phase extraction out of 4000 L of approx. 100 samples. Most of the analytical work on samples collected during the ISPOL experiment was completed during 2007 and early 2008.

Onboard analyses of nutrients, chlorophyll, oxygen and bacterial activity were performed in collaboration with David Thomas and coworkers (University of Bangor, Wales). All samples for land-based analyses were shipped at -20°C to Florida State University for detailed molecular analyses. A special focus is on amino acid enantiomers and ultrahigh resolution mass spectrometry (FTICR-MS).

In addition to in situ sampling, four degradation experiments were performed to study the impact of solar radiation on the formation of persistent DOM in sea ice. These experiments were done in close collaboration with other research groups onboard, covering all major disciplines in biogeochemistry and microbiology. For these experiments unfiltered and sterile-filtered (0.2µm) brine was put into quartz bottles and placed in different depths under snow and on the snow surface. Long-term microbial incubation experiments were completed after 1.5 years.

The drift of the ice floe during the experiment was much slower than anticipated. This facilitated repeated sampling of the water column at the same location for the extraction of large amounts of DOM. However, the spatial coverage of the Weddell Sea was small for the establishment of large scale transport models for DOC. In support of this project, additional sampling was performed during late winter 2006 onboard RV Polarstern (August to October 2006). Objective of our participation on this cruise was to cover the western shelf of Weddell Sea on a large scale for DOC and nutrient analyses with approximately 500 additional samples. This additional data set will fundamentally improve our quantitative models for DOC transport in the Weddell Sea and beyond.



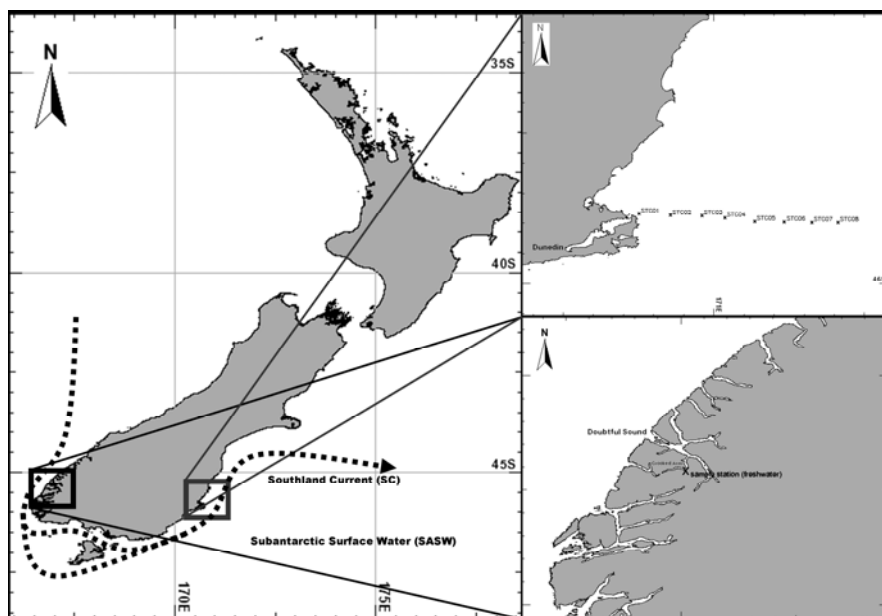
**Figure 1:** The research vessel was anchored to an ice floe between Nov. 28, 2004 and Jan. 2, 2005 at approx. 68°S/55°W. The insert shows the drift of the ice flow during this period.

Additional sampling was performed on the western Weddell Sea Shelf in 2006.

Sea data was the prominence of molecules which appeared to be of terrestrial and thermal origins. To further investigate the mechanisms which would lead to such molecules accumulating in deep ocean water masses, two additional sampling experiments were undertaken. The first included detailed sampling of DOM along a transect across the Subtropical Convergence in Summer and Fall of 2006 and terrestrial DOM in the fjordlands of New Zealand in and along

adjacent coastal margins in June of 2007. The second experiment sampled surface and deep oceanic waters for thermogenic DOM signatures as a part of a CLIVAR cruise in Spring 2008.

The Southern Ocean around New Zealand is characterized by a variety of ocean circulations. The focus of the new study was the connection between outflow from the DOM-rich fjords on the western side of the south island with a narrow band of the Subtropical Convergence (STC) which stretches out from the Otago Coast, Southeast Coast, South Island (Southland Current (SC)) toward the Subantarctic Water (SAW) further offshore. The boundary between the SC on the Southeast coast of the South Island and the SAW is known as the Southland Front (SF). The locations of sampling stations and the major water currents around New Zealand are included in Figure 2.



**Figure 2.** Locations of the sample stations along a transect crossing the Southland Current (SC) into Subantarctic Surface Water (SASW), which is referred to as the Subtropical Convergence (STC) and the freshwater sample station in Doubtful Sound, Fiordland National Park, New Zealand.

The direction of current flow within the STC around southern New Zealand would suggest that a substantial amount of water enriched in terrestrially-derived DOM gets transported from the southwest coast of the South Island via the Southland Current (SC). The combination of high rainfall and dense temperate rainforest on the west coast is responsible for a large input of terrestrially-derived organic matter into the coastal zone. Therefore, terrestrially-derived DOM, or at least the refractory portion of it, could potentially be transported by the Southland Current to the east of the South Island through Foveaux Strait and would be a characteristic of the STC. The concentration of this terrestrially-derived DOM was predicted to decrease toward the Subantarctic Water (SAW). Overall, the terrestrially-derived DOM pool within the STC is potentially influenced by a combination of river discharge, land runoff and transport from the southwest coast, particularly from Fjordland. The Subantarctic Water has a different origin and is barely influenced by the Southland Current; hence DOM in the SAW may exhibit somewhat different characteristics when compared to DOM in the STC.

Quantitation of thermogenic DOM in the oceans was the objective of a third sampling experiment that was a part of a CLIVAR cruise along an oceanographic section from South Africa to Antarctica in early 2008. PI Dittmar and FSU graduate students Jiyoung Paeng and Juliana D'Andrilli participated on CLIVAR cruise I6S at no cost to this project. The participation was fully funded by CLIVAR. Objective of our participation was to obtain samples at high spatial resolution

from the major water masses in the Southern Ocean, including Antarctic Bottoms Water, along a section from South Africa to the Antarctic continent. This region was chosen for this initial study because the ocean off Antarctica is one of the regions least impacted by humans. The subtropical surface ocean off Africa, on the other hand, is strongly influenced by continental runoff and human activity. Several water masses join in the abyssal of the Southern Ocean: deep water that originates from the North Atlantic, and Antarctic intermediate and bottom waters that have been in contact with the atmosphere very recently.

More than 200 samples were collected from the major water masses in the Southern Ocean, and extracted onboard for detailed molecular analysis in PI Dittmar's and co-PI Cooper's laboratories at Florida State University. Our participation on this cruise will extend our database on molecular DOM composition into the Indian Ocean sector of the Southern Ocean and thus facilitate detailed spatial modeling of DOM fluxes and composition.

#### *Chemical Analyses:*

Onboard analyses of nutrients, chlorophyll, oxygen and bacterial activity were performed in collaboration with David Thomas and coworkers (University of Bangor, Wales). Land-based analyses on dissolved organic carbon (DOC) and total dissolved nitrogen (TN) were done with a Shimadzu TOC-V analyzer at FSU. A focus of the second project year was the detailed molecular characterization of DOM. By combining several molecular tracer techniques we were able to identify the fate of ice-algal derived DOM in the different water masses and along diagenetic pathways in the Weddell Sea. The results from this molecular fingerprinting are being integrated into physical models of regional and global ocean circulation, in order to obtain a detailed and quantitative biogeochemical model for DOM cycling.

To obtain a most detailed molecular fingerprint of DOM, a subset of samples was analyzed via Electrospray Ionization Fourier Transform - Ion Cyclotron Resonance - Mass Spectrometry (FT-ICR-MS) at the National High Magnetic Field Laboratory in Tallahassee. With this ultrahigh-resolution mass spectrometry method, several thousand molecules from DOM could be separated and molecular formulae assigned (Koch et al., 2007). To explore this complex dataset, a new statistical approach was developed (Dittmar et al., 2007) and applied to the new Antarctic data set.

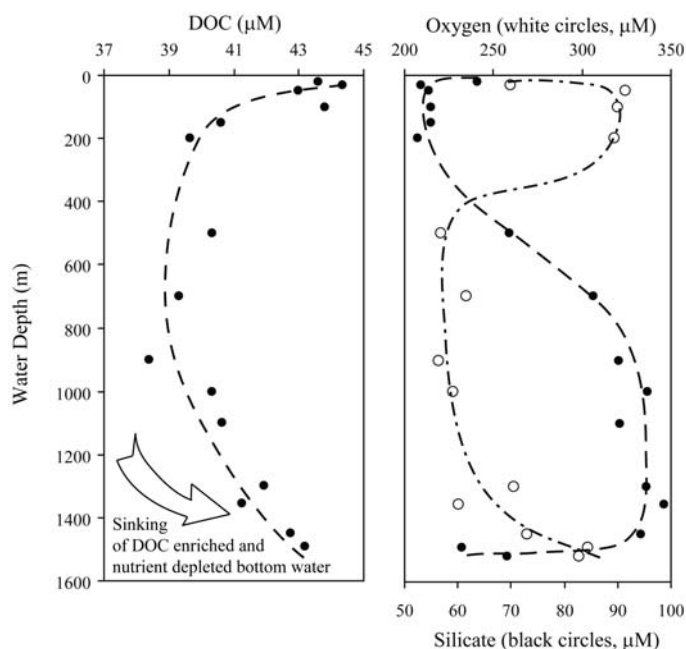
One of the most striking results from the mass spectrometry analysis was the discovery of thermogenic molecules (black carbon) in all analyzed samples (Dittmar and Koch, 2006; Koch and Dittmar, 2006). Aside from the intrinsic relevance of this discovery, thermogenic molecules may also serve as sensitive tracers to identify the source of DOM in the different water masses in the Weddell Sea. FT-ICR-MS cannot be applied as a routine quantitative tool for this purpose, so a new molecular microwave-assisted HPLC technique method was developed for the routine determination of black-carbon derived molecules in marine DOM (Dittmar 2008).

Further development of advanced analytical techniques to support these studies continues. Recent experiments (D'Andrilli et al., submitted) have focused on a comparison of electrospray (ESI) and Atmospheric Pressure Photoionization (APPI) modes for the FT-ICR mass spectrometry analyses. ESI and APPI ionize DOM molecules by very different mechanisms; ESI relies on ionization of polar functional groups like carboxylic acids, while APPI uses high-intensity UV radiation to ionize molecules that are strong UV absorbers, normally those containing double bonds and aromatic rings. These experiments were initiated using samples previously obtained from the ISPOL cruise. The objective is to develop a comprehensive mass spectrometry characterization approach that exploits all the available ionizable molecules in DOM mixtures. This comprehensive approach will be used to characterize samples obtained on the CLIVAR cruise.

## Results and Accomplishments:

### *(1) First indications for a “DOM-pump” in the Weddell Sea*

General biogeochemical data provided first hints on the origin and diagenetic state of the water masses and on the existence of a “DOM-pump” in the Weddell Sea. Weddell deep water (WDW) was characterized by the highest temperatures ( $\sim 0.5^{\circ}\text{C}$ ,  $\sim 600$  m) and represented the oldest water mass being transported southwards from the Antarctic Circumpolar Current. The mineralization of sinking organic particles in the history of this water mass caused nutrient accumulation and oxygen depletion in WDW (Figure 3). The nutrient maximum ( $\sim 1200$  m) is significantly deeper than the temperature maximum of the WDW ( $\sim 600$  m). Hence mixing of WDW with surface and bottom water alone cannot explain the nutrient profiles in the water column. The nutrient increase was rather caused by mineralization of sinking particles. An increasing silicate/nitrate-ratio with water depth reflects the slower mineralization rates of silicate compared to nitrate. Bottom water was the densest water with lowest temperatures ( $-1.7^{\circ}\text{C}$ ) and the highest salinity (34.6). Bottom water was characterized by low nutrient content, high oxygen concentration, and, most importantly in the context of our study, increased DOC concentrations (Figure 3). These profiles indicate the formation of fresh bottom water from biologically active surface water within relatively short periods of time. This is a first indication for DOM being transported from the sea surface into bottom waters in the Southern Ocean.



**Figure 3:** An exemplary water profile during the ISPOL experiment: Increased concentrations of dissolved organic carbon (DOC) in Antarctic Bottom Water (AABW) is the first evidence that DOC is indeed sequestered in the abyssal ocean by processes in the Weddell Sea. Nutrient depletion and increased oxygen concentrations in the bottom water are consistent with this finding. On-going research aims to determine the amount of carbon that is annually sequestered.

The DOC data from the 2006 cruise largely confirm these results. At several stations along the transect a significant increase of DOC concentrations in Antarctic Bottom Water (AABW) were observed, compared to the background concentration of Atlantic Deepwater (ADW). The differences between ADW and AABW were in the range of 0-4  $\mu\text{M}$  DOC. Probably due to the complex hydrography in this region a complex pattern of vertical DOC profiles was observed along the sampled transect. Our DOC profiles from the sea surface down to ADW closely match the recently published DOC data for the Atlantic Ocean north of  $60^{\circ}\text{S}$  (obtained by Dennis Hansell and coworkers, Repeat Hydrography transect A16S, <http://www.rsmas.miami.edu/groups/biogeochem/>

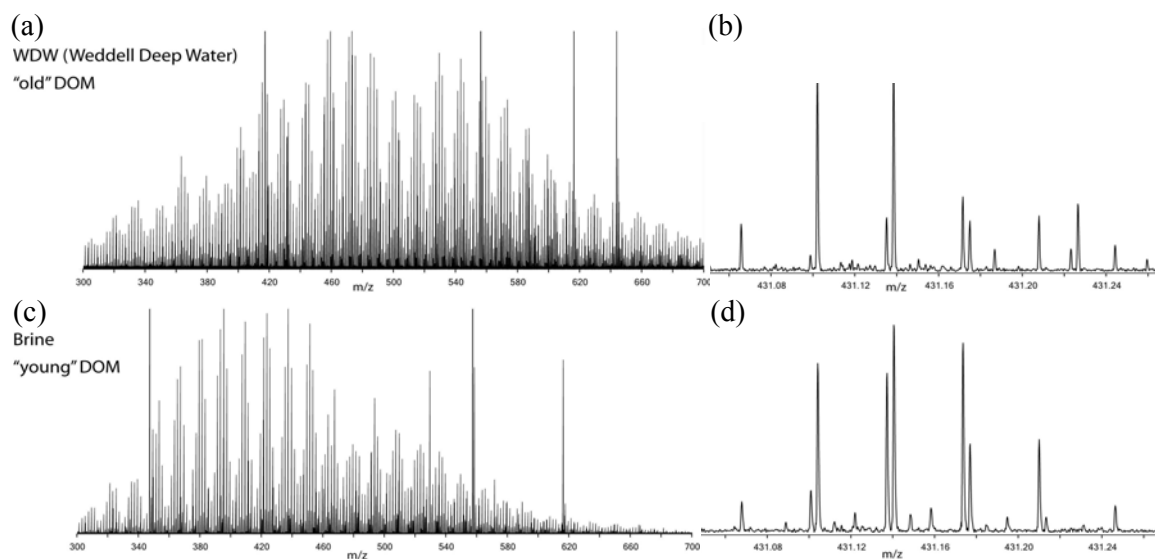
Data.html). Hansell et al., however, did not observe an increase in DOC concentration in AABW for the region north of 60°S.

We propose two hypothesis that can explain the apparent lack of DOC accumulation in AABW north of 60°S: (1) Intrusion of Atlantic water masses reduces the DOC concentration in AABW, so that the difference between AABW and ADW becomes undetectable (a difference of approx. 1  $\mu\text{M}$  DOC would be undetectable). (2) Recently downwelled DOM contains a significant fraction of labile components which are mineralized in AABW during its northward advection.

We are applying two approaches to test these two hypothesis: Hypothesis (1) is being tested by implementing the DOC data into ocean circulation models. Hypothesis (2) is being tested with a molecular fingerprinting approach and incubation experiments.

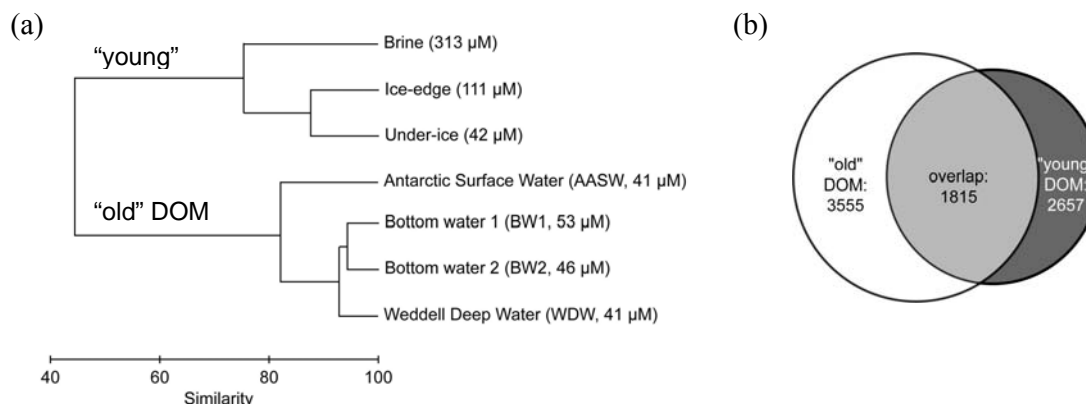
## (2) *Molecular fingerprinting*

To date FT-ICR-MS is the only method capable to resolve intact individual molecules from complex mixtures such as DOM. With help of FT-ICR-MS we resolved more than 3500 individual molecules in Weddell Sea DOM (Figure 4). While the overall patterns were very similar between the samples, some molecules were enriched at the sea surface compared to deep waters whereas others were depleted or absent (Figure 4). We explored these differences between the samples through a new statistical approach (Dittmar et al., 2007) that we adapted to FT-ICR-MS data. Cluster analyses revealed two distinct groups of samples, i.e. “old” and “young” DOM (Figure 5). DOM from sea-ice brine, the ice edge and under the ice (“young”) were very similar in molecular composition, and significantly different from all water column samples (“old”). Bottom Water did not show more molecular similarities with ice-algal DOM than the other deep waters. The difference between the two groups of samples was also manifested in the average elemental composition. Molecules that were present only in “young” DOM were relative rich in nitrogen ( $\text{C/N}=35$ ) and depleted in sulfur ( $\text{C/S}=336$ ) compared to molecules that were present exclusively in “old” DOM ( $\text{C/N}=6080$ ,  $\text{C/S}=45$ ).



**Figure 4:** Ultrahigh-resolution mass spectra (FT-ICR-MS) of two DOM samples: **(a-b)** diagenetically old DOM (Weddell Deep Water) and **(c-d)** freshly produced DOM from sea-ice brine. **(a, c)** Complete spectra (300–700  $m/z$ ) and **(b, d)** very narrow mass range of 1 Da at 431.

Most notable is the fact that aside these observed differences about half of all resolved molecules were present in all samples, independent of their origin and degree of degradation (Figure 5). This finding is most surprising because it infers that a large fraction of deep-sea refractory compounds is being produced in an ice-algae bloom on time scales of days to months. The formation of refractory compounds in an algal bloom seems to be on a similar or shorter time scale than bottom water formation. It is also remarkable that all samples in direct vicinity of the ice had lost a significant fraction of molecules that were present in the deep ocean, which infers that a group of deep-sea compounds seem to be labile at the sea surface.



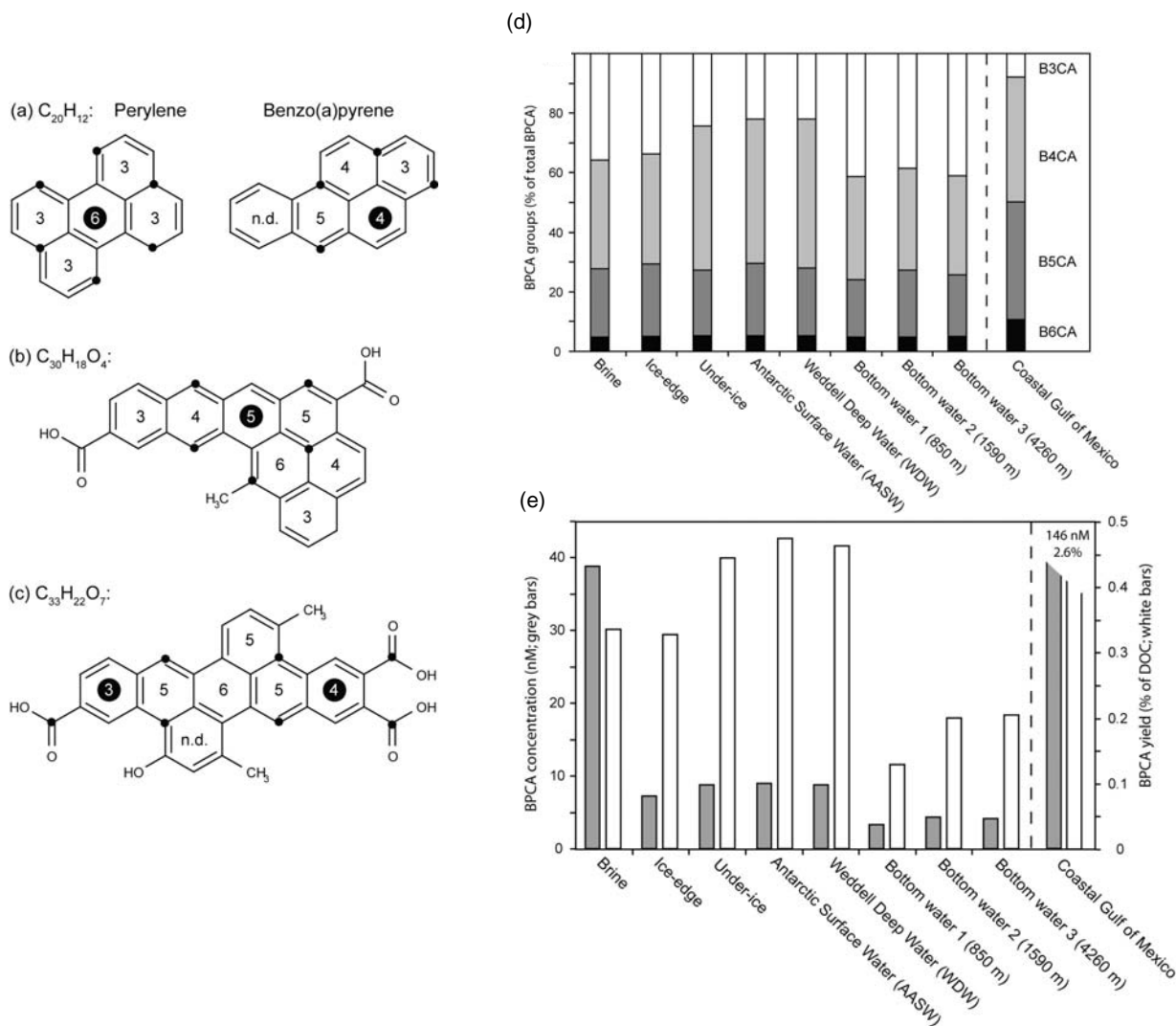
**Figure 5:** (a) Cluster analysis of FT-ICR mass spectra indicate two groups of DOM in the Weddell Sea. (b) More than half of the identified molecules were present in all samples.

More than 200 polyaromatic hydrocarbons (PAHs) were identified in Weddell-Sea DOM, most of them consisting of seven condensed rings with varying numbers of carboxyl, hydroxyl, and aliphatic functional groups. These are unambiguously thermogenic compounds of pyrogenic ("black carbon") or petrogenic origin (Dittmar and Koch, 2006; Koch and Dittmar, 2006). We estimate that  $>2.4\%$  of DOM in the ocean are thermogenic compounds, and their turnover is  $>1.2 \cdot 10^{12}$  mol C per year. This finding has direct relevance for our understanding of global biogeochemical cycles.

Pyrogenic molecules are relatively stable in the environment and may thus be helpful as tracers to identify the flux of DOM in the ocean. FT-ICR-MS cannot be applied on a routine basis for the identification of pyrogenic matter. We therefore developed a new microwave-assisted HPLC technique for the routine determination of black-carbon derived molecules in marine DOM (Dittmar, submitted). Based on the FT-ICR-MS results we propose possible structures for pyrogenic DOM in the ocean (Figure 6). Microwave-assisted oxidation with nitric acid produces a suite of benzepolycarboxylic acids (BPCA) which is consistent with the proposed molecular structures for pyrogenic DOM in the ocean. The BPCA method was tested with different algal cultures, different PAHs and charred materials.

BPCA analysis revealed the presence of black carbon in all DOM samples from the Weddell Sea, whereas algal cultures were free of black carbon. Sea-ice brine had highest concentrations and carbon-normalized yields of black carbon, even though the deposition rate of black-carbon rich aerosols is minimal in the Weddell Sea, compared to other oceanic regions in the world. In the water column a significant trend with higher concentrations at the surface and lower concentrations at the bottom was observed. Thus, bottom water was not significantly influenced by surface waters, whereas Weddell Deep Water (WDW) was enriched in black carbon. We propose the following explanations for this pattern: (1) Sea-ice accumulates black carbon from atmospheric deposition over its approx. two-year cycle between initial formation and melting. (2) The very low black-carbon concentrations in the bottom waters indicate that most of bottom water must have

been formed at an initial phase of sea-ice formation at a time-scale too short for the accumulation of black-carbon deposits. (3) The higher concentration in Weddell Deep Water and Surface Water indicate a linkage with the sea ice. Sinking of black-carbon rich particles during melting and dissolution in the water column is a potential mechanisms to may explain the observed pattern.



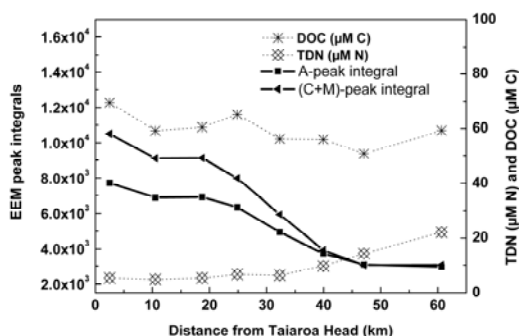
**Figure 6:** Benzenepolycarboxylic acid (BPCA) analysis of Weddell-Sea DOM.

(a) PAHs used for method validation, (b-c) likely structures of black-carbon derived molecules in Weddell-Sea DOM, deduced from BPCA and FT-ICR-MS analyses. The numbers in the aromatic cycles indicate the number of carboxylic groups produced during nitric acid oxidation. The dark numbers are the most commonly found BPCAs, the marked positions surrounding these rings are being oxidized to carboxylic groups during nitric acid oxidation. (d) The composition of BPCA in marine DOM (B3CA is the sum of benzenetricarboxylic acids, B4CA benzenetetracarboxylic acids, B5CA benzenepentacarboxylic acid, and B6CA benzenhexacarboxylic acid). (e) Concentration and carbon-normalized yields of BPCA in marine DOM. For comparison, a terrestrially influenced DOM sample from the coastal Gulf of Mexico is included.



### (3) *Linking Terrestrial DOM Sources and Oceanic Sinks in the Subtropical Convergence off the South Island of New Zealand*

The position of the STC at the Southland Front was clearly identified from the continuous salinity and temperature measurements and was found to be consistent with previous reports. The DOC concentration showed only small variations across the STC with values ranging between 55 and 70  $\mu\text{M C}$  (Figure 7). TDN concentrations were constant in the near shore water and in the SC, but increased continuously from 30 km offshore toward the SASW. This increase in the TDN content may reflect relatively high nutrients in the SASW, which is comparable to a steady increase in nitrate levels measured across a similar transect from coastal to SASW in a previous study. (Coble, 1996). In contrast to DOC, the fluorescence intensities of the terrestrial component significantly decreased across the STC transect (Figure 7). Clearly, coastal water and water within the SC exhibited higher CDOM levels than SASW.



**Figure 7.** EEM fluorescence peak integrals, DOC and TDN values for surface water samples collected across the STC in January 2006.

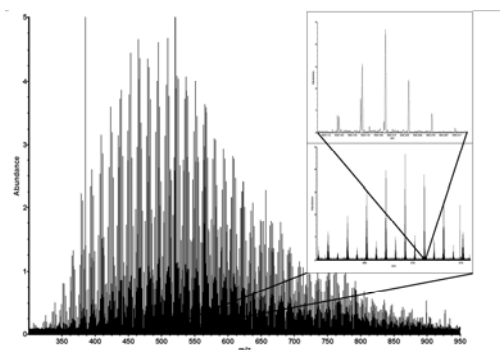
Analyses of lignin derivatives as biomarkers for terrestrially-derived DOM were used to characterize the terrestrially-derived DOM pool across the STC. DOM was obtained from surface water samples were collected during National Institute of Water and Atmosphere (NIWA) cruises using the University of Otago research vessel *R/V Munida* at 8 sample stations along a transect crossing the STC and reaching Subantarctic Water 60 km offshore. The individual amounts of all eight lignin derivatives determined using the CuO oxidation method decreased across the STC (Table 1). The acid/aldehyde ratios ((Ad/Al)/v) were high, which is an indication of a high degree of decomposition caused by propyl side chain oxidation (e.g., white-rot decay). Additionally, the ratio of non-methoxylated phenols (p- hydroxyphenols) to methoxylated phenols (vanillyl and syringyl phenols) (P/(V+S)) is also a measure for the degree of degradation. Demethylation caused for example by brown-rot decay may lead to the degradation of methoxylated phenols resulting in an increase in this ratio. The samples collected across the STC showed high values for this P/(V+S) ratio, which was also indicative of a high degree of degradation.

Clearly, the amount of recognizable lignin (Sum8 in Table 1) is very low in all samples across the STC indicating either a very low amount of lignin present or highly degraded lignin, which is no longer recognized by the analytical technique as lignin. Furthermore, the total recognizable lignin levels also decreased from the coastal zone (sample STC01) across the STC and reached a minimum value in the SAW (sample STC08), which was 10-fold lower compared to the water closest to the coast.

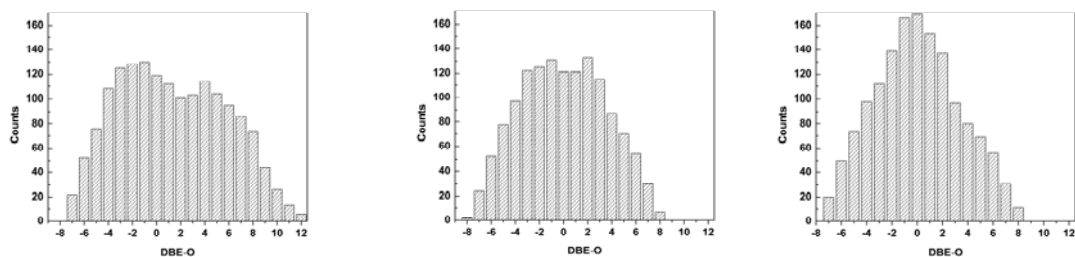
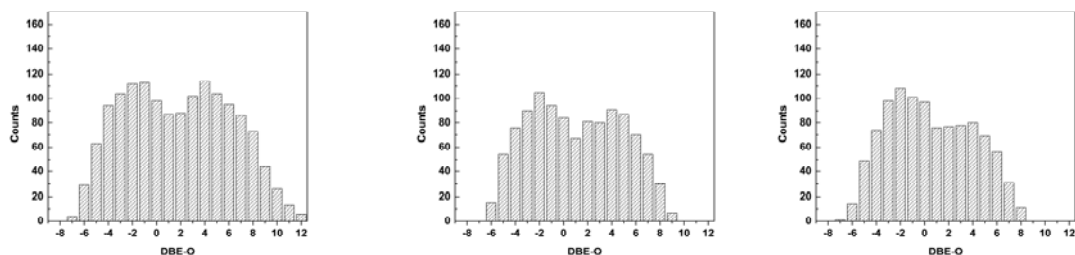
Figure 8 contains a mass spectrum from sample station STC01 collected in January 2006. This spectrum is typical of samples from the STC that were analyzed in this study and illustrates the complexity of mass spectrometric data provided by ultrahigh resolution FT-ICR MS.

**Table 1:** Lignin analysis across the Subtropical Convergence (STC)

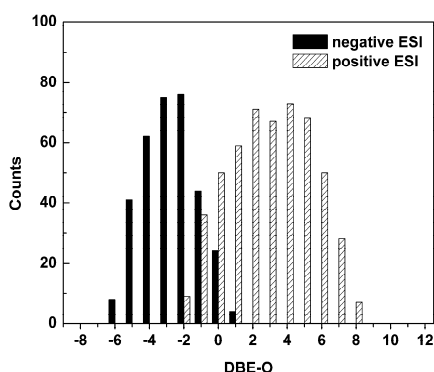
sample	S/V	C/V	(Ad/Al)v	P/(V+S)	Sum8 (nM/mg)
STC01	n.d.	n.d.	1.57±0.13	2.06±0.24	1.59±0.16
STC04	n.d.	n.d.	1.59±0.23	2.83±0.88	0.87±0.23
STC08	n.d.	n.d.	2.35±0.23	4.57±0.92	0.14±0.01

**Figure 8:** Ultrahigh resolution FT-ICR mass spectrum for a sample collected across the Subtropical Convergence at station STC01 in Austral summer (January) 2006.

A clear trend in the decrease of unsaturated CHO compounds was observed across the STC in Austral summer (Figure 9) and winter (Figure 10). This trend is reflected in the loss of compounds with high double bond equivalencies (DBE). The DBE factor can be calculated once the chemical formula is known and represents the number of double bonds and rings in the compound. We prefer to express unsaturation by subtracting the number of oxygen atoms from the DBE value, yielding a DBE-O factor. Compounds with DBE-O values ranging from 9-12 were significantly depleted in DOM from samples in the eastern portion of the convergence.

**Figure 9:** DBE-O frequency plots for CHO-compounds across the Subtropical Convergence at sample stations STC01 (left), STC04 (middle) and STC08 (right) in Austral summer.**Figure 10:** DBE-O frequency plots for CHO-compounds across the Subtropical Convergence at sample stations STC01 (left), STC04 (middle) and STC08 (right) in Austral winter.

To better describe changes in DOM characteristics in surface waters along the near- to off-shore transect, molecular formulae were assigned from the mass spectra of all water samples using both negative and positive mode ESI. Only 3 % of the CHO compounds determined for the negative mode ESI but a remarkable 60 % of the CHO compounds determined for the positive mode ESI were unique for the coastal DOM (STC01) compared to the off-shore DOM sample (STC08). The ability to efficiently deprotonate in the positive mode ESI and the absence of all these compounds in negative mode ESI suggest a very low level of carboxylation. The low oxygen content of the presented molecular formulae also suggested that these compounds are unlikely to be saccharides. Compared to the bulk of DOM, the compounds analyzed by positive ESI and present exclusively in the coastal surface waters showed a relatively high degree of unsaturation, which is consistent with the abundance of lignin observed in this sample. The presumably aromatic character of these compounds may also explain the comparatively strong fluorescence exhibited by the coastal surface water sample. A similar relationship between fluorescence intensity and unsaturation observed by ESI-FT-ICR-MS has been previously reported in a study of coastal outwelling in a Brazilian mangrove area. Interestingly, more presumably refractory compounds were detected in positive ESI mode suggesting a higher degree of carbon unsaturation compared to the negative mode ESI (Figure 11).

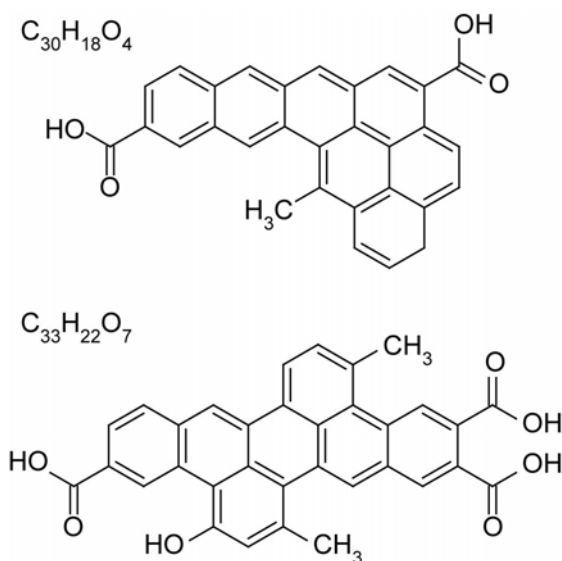


**Figure 11.** DBE-O frequency plot of the proposed refractory DOM pool in all samples collected in January and August 2006 across the STC and measured using negative and positive ESI modes.

#### **(4) Identification of Thermogenic DOM in the South Atlantic**

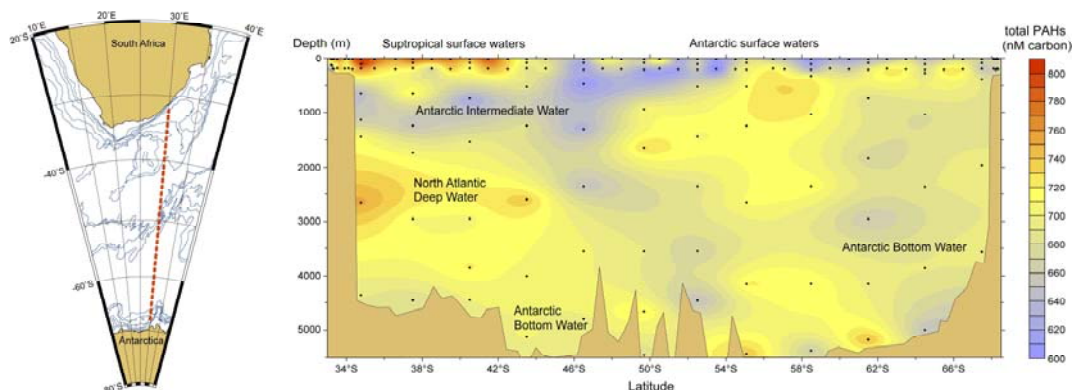
First FT-ICR-MS analysis of marine DOM yielded surprising results. In the abyssal ocean off Antarctica molecular structures were found that cannot be produced by any known organism (Dittmar and Koch, 2006). The large polyaromatic cores (PAHs, Figure 12) of these structures were undoubtedly derived from thermal processes. At this point, the implications for global biogeochemical cycles remained unclear, because the quantity of thermogenic DOM could not be determined. FT-ICR-MS is unsurpassed in resolving individual molecules from complex mixtures, but the method is considered at best semi-quantitative. To determine the concentration of the newly discovered fused ring systems in marine DOM, a quantitative method, originally developed for coal and soil analyses, was adapted for the quantification of thermogenic structures in DOM (Dittmar, 2008). This method provides only basic structural information, but it is quantitative, and therefore complements FT-ICR-MS. The method was first employed along an oceanographic section from South Africa to Antarctica in 2008. This region was chosen for this initial study, because the ocean off Antarctica is one of the regions least impacted by humans. The subtropical surface ocean off Africa, on the other hand, is strongly influenced by continental runoff and human activity. Several water masses join in the abyssal of the Southern Ocean: deep water that originates from the North

Atlantic, and Antarctic intermediate and bottom waters that have been in contact with the atmosphere very recently.



**Figure 12:** An example of likely structures of thermogenic DOM in the deep ocean (Dittmar and Koch, 2006). The structure was proposed based on exact molecular masses obtained via FT-ICR-MS. The same fused ring structures are likely a major component of petroleum asphaltene (Ruiz-Morales and Mullins, 2007). In the case of asphaltene, the carboxylic functional groups found in DOM are substituted by alkyl and other hydrophobic groups.

First results from this research (Figure 13) show a surprisingly homogenous distribution of thermogenic DOM in the deep ocean. The concentration of carbon that resides in thermogenic PAHs varied between 610 and 800 nM carbon, or approximately 2% of DOM (on a carbon basis). To explain this lack of major gradients in the deep ocean, thermogenic DOM must behave virtually inert in this environment. At the sea surface, on the other hand, continental influence caused elevated concentrations in subtropical waters, whereas cold Antarctica waters were depleted in thermogenic DOM. This depletion was preserved in Antarctic intermediate water that originates from the sinking of surface waters off Antarctica. The observed gradients at the sea surface indicate that some of the source and sink terms are associated to surface processes. River runoff and soot deposition are potential candidates for surface-related sources. Photochemical reactions or affinity to sinking particles may explain the observed surface depletion off Antarctica.



**Figure 13:** The distribution of thermogenic DOM in the Southern Ocean, expressed as the concentration of total PAHs (nmol carbon per liter). The likely structure of these highly functionalized PAHs is shown in Figure 1. The water samples were obtained on CLIVAR cruise I6S (4 February - 17 March, 2008) onboard research vessel Roger Revelle. Thermogenic DOM was quantified as described in Dittmar (2008).

At present, humans are the largest source of combustion products on earth. It is therefore surprising that the oldest water masses which are otherwise not enriched in industrial products (such as the Freon CFC-11, CLIVAR), in particular North Atlantic deep water, showed highest concentrations of thermogenic DOM. The younger water masses which are enriched in freons (Antarctic bottom and intermediate waters) are not particularly enriched in thermogenic DOM. The preindustrial background clearly predominates over more recent processes in the deep ocean. Human activity probably had a minor impact on the more than one Peta ( $10^{15}$ ) mole carbon that resides in the deep ocean as thermogenic DOM. The presumably inert character makes this relatively small fraction of DOM a relevant component in the pre-industrial global carbon cycle.

The question remains whether thermogenic DOM is derived primarily from wildfires on land or from thermal processes in deep sediments. It is intriguing that the same polyaromatic cores that we found in marine DOM (Figure 12) are abundant in petroleum asphaltene. This structural similarity indicates a common origin which would imply a novel link between fossil sedimentary organic carbon which is locked into the rock cycle and active cycles on earth's surface. Sharp land-ocean gradients of thermogenic DOM point towards continental runoff as another potential source (Dittmar, 2008). Radiocarbon dating of this compounds class in the deep sea may provide final proof of the formation processes behind thermogenic DOM.

#### **(5) Comparison of Ultrahigh Resolution Mass Spectra of Marine DOM Obtained by Electrospray and Atmospheric Pressure Photoionization**

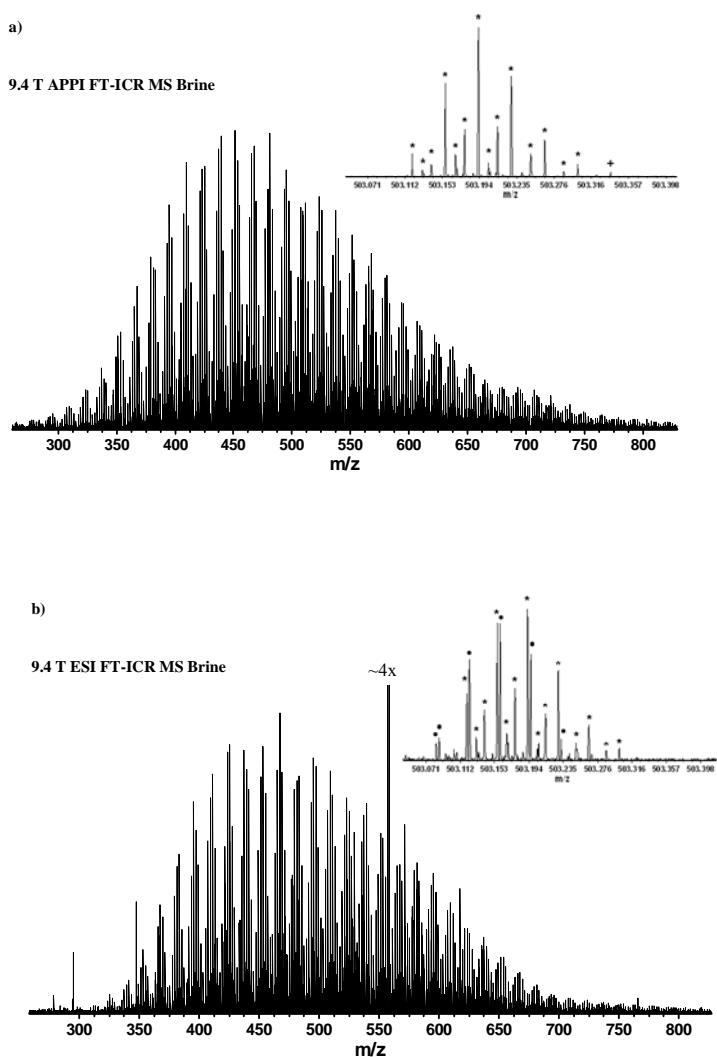
Electrospray Ionization (ESI) and Atmospheric Pressure Photoionization (APPI) are two of the most popular soft ionization techniques that, when coupled with FT-ICR-MS, are ideal for DOM characterization. Analytes must be ionized before MS analysis and ESI and APPI are capable of producing largely intact ions from a non-selective source with minimal fragmentation. ESI has been the predominant ionization method of choice for FT-ICR-MS analysis of terrestrial and marine DOM.

ESI ionizes the polar species in the analyte solution then transfers the ions to the gas phase for MS analysis at atmospheric pressure. ESI ionizes polar compounds with amine ( $-\text{NH}_2$ ) and carboxylic acid ( $-\text{COOH}$ ) functional groups and has numerous advantages in biogeochemistry. It is capable of ionizing compounds over a large mass range ( $10 < m/z < 3000$ ), can create positively or negatively charged molecular ions, and can easily be coupled with ultra-high resolution mass spectrometry. Even though this method is widely used and labeled as non-selective, it tends to be selective by ionizing only the molecules that contain both polar and non-polar regions of DOM analytes. However, recent DOM characterization experiments using ESI FT-ICR MS are initiating further inquiries regarding the capability and limitations of the ionization technique, specifically its low efficiency ionization of non-polar species.

Figures 14a and 14b show APPI and ESI 9.4T FT-ICR mass spectra of the Weddell Sea Brine DOM extract. Clearly, both spectra have very distinct Gaussian shaped distributions ranging from 300-800  $m/z$ . It is very difficult to determine which ionization technique provides a wider range of ionizable molecules, so a portion of the spectra was chosen for further visual interpretation ranging within 1Da of 503  $m/z$  (inset of Figures 14a and b). The mass error between the theoretical formula and the measured masses is  $< 550$  ppb and the resolving power is  $> 500,000$  at 500 Da. From 503.071-503.398  $m/z$  there are 20 assigned formulas seen in ESI and only 15 for APPI. The Figure 14a and b insets show the common and uncommon spectral peaks of ESI and APPI. ESI produces a range of CHO compounds ranging from  $\text{O}_7\text{-O}_{13}$  whereas it is  $\text{O}_6\text{-O}_{12}$  for APPI. The major difference between the two is the five unique molecular formulas assigned from ESI all containing one sulfur atom. Within this 1Da window at 503  $m/z$ , not one sulfur atom is assigned to

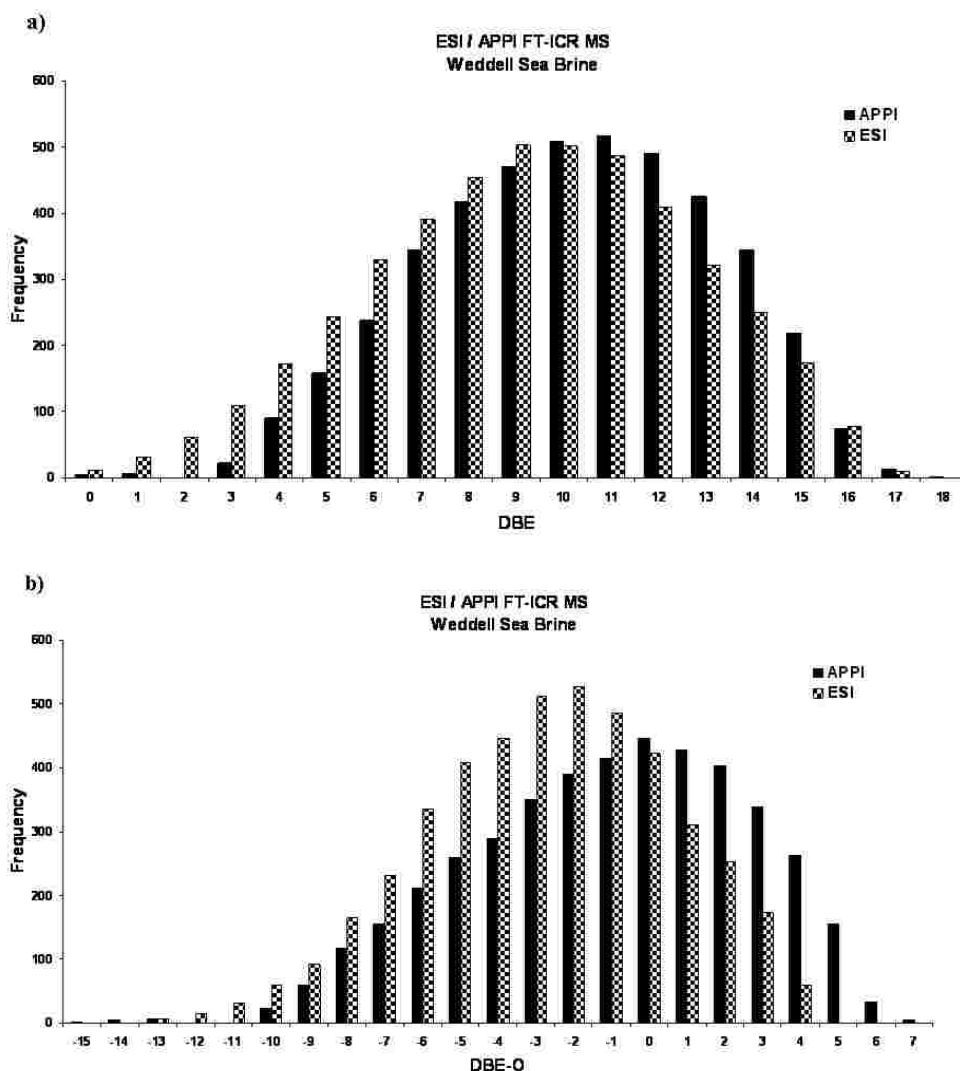
any molecular formula for APPI. This trend is generally consistent across the entire spectrum, as we have monitored three other windows from 400-600  $m/z$  to confirm our hypothesis that the sulfur compounds present within marine DOM exist either as a polar group, i.e. sulfite or sulfate, or are a part of a larger polar species and therefore are not ionized efficiently by APPI.

Figures 15a and 15b compare the APPI and ESI data for DBE and DBE-O. The distribution over DBE values nearly identical in Figure 15a, but the most abundant DBE values are different for APPI and ESI. In APPI, the maximum is reached at a DBE value of 11 whereas 9 is the ESI maximum DBE value. The same trend is seen in Figure 15b (although the shapes of the distributions are obviously dissimilar) where there is a shift to more abundance positive DBE-O values for APPI describing fewer highly unsaturated aromatic compounds.



**Figure 14:** Weddell Sea Brine mass spectra. (a) APPI FT-ICR mass spectrum and (b) ESI FT-ICR mass spectrum at 9.4 T. Insets of both spectra are within 1Da at 503 $m/z$ .

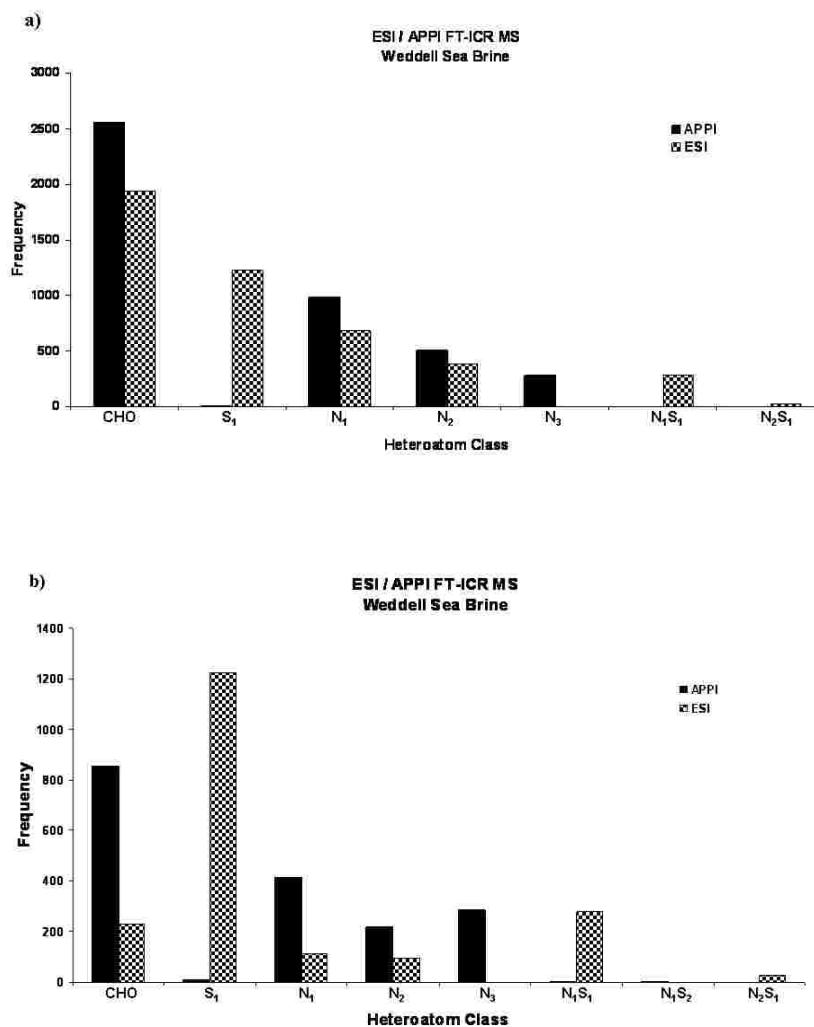
- \* = Common molecular formulas
- + = Unique APPI molecular formulas
- = Unique ESI molecular formulas



**Figure 15:** Weddell Sea Brine (a) ESI / APPI Double-Bond Equivalence distribution and (b) ESI / APPI Double-Bond Equivalence minus Oxygen distribution. Both a) and b) show the entire Brine data set including all assigned molecular formulas.

Heteroatom (N and S) classifications summed for all the formulas assigned for Brine DOM using both ionization techniques are displayed in Figure 16a. The classes describe the heteroatom content of molecules, e.g., the  $S_1$  class contains one sulfur atom with the remaining atoms in the molecule consisting of carbon, hydrogen, and oxygen. For molecules observed by both techniques, the CHO class is the most abundant. APPI more efficiently ionized all chemical classes than ESI except for compounds containing sulfur. Similarly, Figure 16b represents the non-matching data by class. It is informative that of the molecules observed by only one technique, the class most frequently detected is different for APPI (CHO) than for ESI ( $S_1$ ). This observation supports our

contention that combined ESI and APPI analyses are necessary to provide a complete molecular representation of marine DOM.



**Figure 16:** Heteroatom class distribution for Weddell Sea Brine. Left: All assigned molecular formulas for ESI and APPI. Right: Formulas observed by only ESI or only



## Conclusions

1. During bottom water formation in the Weddell Sea significant amounts of refractory DOC are carried into the deep ocean. North of 60°S, however, Antarctic Bottom Water (AABW) is not enriched in DOC compared to Atlantic Deep Water. Circulation models will be established in the third project year to quantify the amount of DOC being sequestered in the Weddell Sea and to determine whether the absence of DOC accumulation in AABW can be explained via intrusion of DOC-poor Atlantic waters.
2. The ice-covered surface Weddell Sea is a source and probably also a sink for refractory DOM. More than half of the refractory compounds found in the deep-sea are produced within days to month in an ice-algae bloom. A different fraction of deep-sea DOM, however, seems to be labile at the sea surface.
3. A decrease of unsaturated CHO compounds was observed across the STC off the coast of the South Island of New Zealand in Austral summer and winter. This trend is reflected in the loss of compounds with high double bond equivalencies (DBE). Compounds with DBE-O values ranging from 9-12 were significantly depleted in DOM from samples in the eastern portion of the convergence. Photochemical effects are hypothesized as the source of these observations.
4. Sea-ice accumulates black carbon from atmospheric deposition over its approx. two-year cycle between initial formation and melting. The very low black-carbon concentrations in bottom waters indicate that most of DOC is carried into the deep sea from ice-free polynias or during an initial phase of sea-ice formation in early winter. Sinking of black-carbon rich particles during melting and dissolution in the water column is the probable reason for enhanced black carbon concentrations in intermediate waters from the Atlantic.
5. The preindustrial background clearly predominates over more recent processes in the deep ocean. Human activity probably had a minor impact on the more than one Peta ( $10^{15}$ ) mole carbon that resides in the deep ocean as thermogenic DOM. The presumably inert character makes this relatively small fraction of DOM a relevant component in the pre-industrial global carbon cycle.
6. Both electrospray and atmospheric pressure photoionization are necessary to get a complete and comprehensive representation of DOM molecules in these complex samples. Nearly all the sulfur species were detected by ESI, thus supporting our hypothesis that the majority of sulfur-containing compounds in Brine and WDW exist as a polar species. We can deduce that the sulfur-containing compounds present in Brine and WDW must be polar if less than 1% are detected by APPI. Also, there were non-polar species more efficiently ionized by APPI that were found to be significant and cannot be neglected. These results emphasize the need to combine the data from both ionization techniques for a more accurate representation of marine DOM.

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